

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

William Pollack

Application No.: 09/660,862

Filed: September 13, 2000

For: METHOD OF MANUFACTURING  
IMMUNE GLOBULIN

Customer No.: 20350

Confirmation No. 7947

Examiner: V. Ford

Technology Center/Art Unit: 1645

PRELIMINARY AMENDMENTCommissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, William Pollack, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I am currently chairman and chief executive officer of Atopix Pharmaceuticals Corporation, the assignee of the subject application.

3. I, Dr. Pollack, graduated from the Imperial College of Science and Technology at London University with a B.Sc degree in chemistry and physics. I received a M.Sc degree in physiology and biochemistry from St. Georges Hospital Medical School at London University and a Ph.D. in immunology and immunochemistry from Rutgers University. A copy of my curriculum vitae is attached hereto as Exhibit A.

4. I am the named and true inventor of the above-referenced patent application. I have read and am familiar with the contents of the patent application. In addition, I have read the final Office Action, dated September 8, 2004, received in the present case. It is

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my understanding that the Examiner believes that the Laursen *et al.* patent, U.S. Patent Number 6,281,336, in combination with Flaa *et al.*, patent, U.S. Patent Number 6,165,981 makes the invention of the present application obvious.

5. With this application, I claim a method of manufacturing a highly purified IgG4 immune globulin preparation. The method comprises the steps of adjusting plasma to a pH of 6.5 and a conductivity of between 3.5-6 millisiemens, and contacting the plasma with an anion exchange resin followed by a cation exchange resin to separate the IgG4 from other IgG subtypes and obtain a final effluent that comprises IgG4 essentially free of other IgG subtypes.

6. The Laursen *et al.* method, unlike the method of the present application, does not result in an immunoglobulin preparation comprising IgG4 that is essentially free of other IgG subtypes. The Laursen *et al.* method, instead, results in a purified stable IgG gamma globulin preparation (also referred to as IVIG) containing all IgG subtypes including IgG1, IgG2, IgG3, and IgG4. Laursen *et al.* does not suggest or teach separation of IgG4 from other IgG subtypes to arrive at an IgG4 that is essentially free of other IgG subtypes. Laursen *et al.* neither suggest nor teach that their purification method results in pure IgG4 essentially free of all other subtypes, nor, in my opinion, could the purification system described in Laursen *et al.* result in IgG4 essentially free of other IgG subtypes.

7. The Office Action asserts that Laursen *et al.* teach a method of producing IgG4. This assertion appears to be based on a table showing IgG subtype distribution of total IgG preparations and a description of how the values in the table were determined. See, *e.g.*, Laursen *et al.*, Table at columns 17 and 18, and column 20, lines 9-15. In my opinion, the passages cited by the Office Action do not demonstrate the claimed production of IgG4 that is essentially free of other IgG subtypes and do not demonstrate methods of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

8. Laursen *et al.* disclose methods to purify total IgG from other plasma components. Laursen *et al.* teach adjustment of plasma protein pH to a pH less than 6.0, preferably pH 5.4. The total IgG proteins are solubilized using PEG precipitation which also removes viral particles. The soluble IgG proteins are then subjected to anion exchange chromatography followed by cation exchange chromatography. Laursen *et al.* teach that the total

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IgG fraction binds to the cation exchange resin at a pH less than pH 6.0. A salt gradient is used to displace the total IgG fraction, including IgG4 from the cation exchange resin. See, e.g., Laursen *et al* at column 6, lines 61-64; column 8, lines 32-37 and 45-60. The cited Table at columns 17 and 18 shows the IgG subtype distribution found in the purified total IgG preparation made by the above-described methods of Laursen *et al*. for comparison to commercially available IgG products. The Laursen *et al*. total IgG product includes 98.5% IgG1, IgG2, or IgG3 subtypes and only 1.5% IgG4 subtype. Thus, the disclosure of Laursen *et al*. do not teach methods of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

9. Laursen *et al*. disclosed the subtype distribution of their total IgG product in the Table at columns 17 and 18. Laursen *et al*. determined the subtype distribution using a quantitative method called Mancini immunodiffusion. See, e.g., Laursen *et al*., at column 20, lines 6-15. Mancini immunodiffusion is a semi-quantitative technique used for quantitative analysis of plasma proteins and in my opinion, is of no value for the fractional separation of proteins, particularly immunoglobulins from one another. In fact, as performed, the assay adds additional IgG antibodies to the total IgG sample of Laursen *et al*. solely for quantification purposes. A brief description of the Mancini immunodiffusion technique follows. To determine the subtype distribution within the total IgG preparation, a sample of the total IgG preparation is added to agar containing an antibody specific for one of the four IgG subtypes (an anti-IgG subtype antibody). If an IgG subtype is recognized by the antibody in the agar, the IgG subtype binds to the antibody forming an IgG subtype-antibody-complex demonstrated as a visible turbid ring of precipitation in the agar. The area of precipitation is proportional to concentration of the IgG subtype, and thus the amount IgG subtypes within the total IgG sample can be determined. The specific IgG subtypes do not require separation from other components of the total IgG sample for formation of the detectable IgG subtype-antibody-complex and, therefore, separations of individual IgG subtypes are not performed. The end product of a Mancini immunodiffusion assay is an agar gel containing a mixture of unbound IgG subtypes from the original sample, unbound specific antibody used for detection and an IgG subtype-anti-IgG antibody complex. For example, if the assay was used to determine a level of IgG4 in the total IgG sample, the end product would be agar containing a mixture of unbound IgG1, 2, 3 and 4 proteins, unbound anti-

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IgG4 antibody and IgG4 antigen-antibody complexes. A schematic drawing showing a Mancini immunodiffusion assay is included as Exhibit B. It should be recognized that for the assay described in Laursen *et al.* the antigen is the total IgG preparation and thus, a mixture of at least four types of IgG protein. Moreover, an IgG4-anti-IgG4 complex is not IgG4 essentially free of other IgG subtypes because the IgG1, 2 and 3 subtypes are also present within the agar matrix. Thus, while the assay can be used to determine a level of a particular IgG subtype, such as IgG4, the assay does not provide a method of separating IgG4 from other IgG subtypes to produce IgG4 that is essentially free of other IgG subtypes.

10. The Office Action appears to assert that modification of Laursen *et al.* to arrive at the claimed method of manufacture of IgG4 that is essentially free of other IgG subtypes is obvious. In my opinion, the modification of the methods of Laursen *et al.* suggested by the Office Action would render the both the methods and the disclosed total IgG product of Laursen *et al.* unsuitable for its intended purpose.

11. Laursen *et al.* disclose manufacture of total IgG with the intent to administer the product to patients that require treatment of diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood, including *e.g.*, primary and secondary agammaglobulinemia, Wiskott-Aldrich syndrome, severe combined immunodeficiency, treatment of autoimmune diseases, and treatment of certain patients with immune conditions. See, *e.g.*, Laursen *et al.* at column 15, lines 8-47. A purified IgG4 preparation would not be useful to treat disease that require supplementation of total IgG. Therefore, in my opinion, modification of the methods of Laursen *et al.* to arrive at the claimed methods to manufacture IgG4 that is essentially free of other IgG subtypes would render the methods of Laursen *et al.* unfit for their intended purpose of manufacturing a total IgG product for treatment of diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood.

12. Laursen *et al.* specifically disclaim adjustment of plasma pH to values greater than pH 6.0. See, *e.g.*, Laursen *et al.*, at column 5, lines 12-17. A pH below 6.0 is required to optimize a subsequent PEG precipitation step. The PEG precipitation step is used to stabilize the total IgG fraction, while precipitating out infectious viral particles. Laursen *et al.* at column 6, lines 1-6. Increasing the pH of plasma as specifically disclaimed by Laursen *et al.*

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would most likely decrease the effectiveness of the PEG precipitation step by e.g., decreasing the stability of the IgG and/or reducing the effectiveness of virus particle removal. Either result, in my opinion, would render the Laursen *et al.* methods unsuitable for production of a stable, virus-free, purified total IgG component of blood for safe administration to patients with diseases or conditions that benefit from replacement or supplementation of the total IgG component of blood and thus, unsuitable for intended purpose of the methods.

13. Further, Larsen *et al.* in view of Flaa *et al.* does not make obvious the manufacturing method of the present application, as the secondary reference of Flaa *et al.* does not address the deficiencies in the Larsen *et al.*

14. Flaa *et al.* teach only solutions for stabilizing purified proteins and do not teach any methods for separating proteins, including immunoglobulins, and in particular IgG subtypes such as IgG4.

15. In view of the foregoing, it is my scientific opinion that, after reading the above mentioned references, the presently claimed method is novel and unobvious over the cited art. Based on the disclosure of the cited references, one of skill in the art would not be motivated to make the purified IgG4 preparations using the method of the present application. Therefore, Laursen *et al.* either alone or in combination with Flaa *et al.* does not make the invention of the present application obvious.

The declarant has further nothing to say.

Date:

12-22-04

By:

William Pollack

William Pollack, Ph.D.

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**CURRICULUM VITAE  
OF  
DR. WILLIAM POLLACK**

**Education**

Imperial College of Science & Technology. (London University, England).  
B.Sc., A.R.C.S., (Major Chemistry & Physics.)

St. George's Hospital Medical School. (London University. England). M.Sc., (Physiology & Biochemistry). F.R.C.Path.

Rutgers - the State University of New Jersey.  
Ph.D. (Immunology & Immunochemistry). Dissertation thesis: *"A study of the Factors Affecting the Zeta-Potential and Hemagglutination with Human Iso-Antibodies"*.

**Professional History**

**1994-Present**      Atopix Pharmaceuticals Corporation  
Carlsbad, California, 92008  
Chairman & C.E.O.

**1981-1985**        The Purdue Frederick Co.  
Norfolk, Conn. 06856  
Vice President, R/D  
Member of Executive Committee.

**1956-1981**        Ortho Diagnostics Systems, Inc.  
Raritan, NJ 08869 (A Johnson & Johnson Co.)  
Vice President and Member of Board of Directors  
Director of Research.

**1954-1956**        Royal Columbian Hospital  
British Columbia, Canada.  
Director of Blood Bank & Clinical Laboratories.

**1948-1954**        St. George's Hospital Medical School, London, England

**1943-1946**        Royal Navy, Honorable Discharge  
Lieutenant, Service in North Atlantic and Pacific

## **Academic Appointments**

- 1974-1985** Associate Adjunct Professor.  
University of Medicine & Dentistry of New Jersey.  
(Previously Rutgers Medical School)
- 1968-1981** Associate Clinical Professor of Pathology  
College of Physicians and Surgeons  
Columbia University, New York City, NY.

## **Awards and Honors**

- 1969** Karl Landsteiner Award of the American  
Association of Blood Banks.
- 1976** John Scott Award, Philadelphia Board of  
Directors of City Trusts.
- 1978** XXXI Annual Gibson Lecturer, Columbia  
Presbyterian Medical Center, New York City.
- 1979** Joseph Bolivar-DeLee Humanitarian Award.  
Chicago, Illinois.
- 1980** Albert and Mary Lasker Clinical Medical Research  
Award.
- 1987** Award from New York State Perinatal Society for  
Unique Contributions to Maternal & Child Health.
- Listed in: Who's Who in America, Who's Who in  
the World and American Men and Women of  
Science.

### **Present and Past Professional and Honorary Societies**

American Association for the Advancement of Science

American Association of Blood Banks

American Association of Immunologists

American Society of Clinical Pathologists

American Society of Hematology

British Society of Immunology

Harvey Society

International Society of Blood Transfusion

New York Academy of Medicine

New York Academy of Science

Reticuloendothelial Society

Sigma Xi



### Partial Bibliography

- 1958 Levine, P., Stroup, M. and Pollack, W., *Human Blood Groups*. In: *Bacterial and Mycotic Infections in man* by Dubos, R.J. 3rd Ed.
- Pollack, W., Reiss, A.M. and Treacy, M. *Observations on the Direct Coombs Test on Cord Erythrocytes after the Addition of Complement*. Vox Sanguinis 3: 442-448
- 1959 Hill, G., Pollack, W. and Levine, P. *A Critical Evaluation of a Rapid 'Broad Spectrum' Compatibility Test*. In: International Society of Blood Transfusion Proceedings of the 7th Congress, Rome, 1958, Basel; S. Karger, pp. 341-343
- Pollack, W., Reiss, A.M. and Treacy, M. *The Use of Complement in the Serological Diagnosis of Hemolytic Disease of the Newborn*. In: International Society of Blood Transfusion, Proceedings of the 7th Congress, Rome, 1958. Basel; S. Karger, pp 519-522
- 1960 Hill, G. and Pollack, W. *Evaluation of Antibody Detection Techniques*. Int. Soc. Blood Transfusion. 8th Congress, Tokyo, 1960. Basel/New York; S. Karger, pp. 230-232
- 1961 Levine, P., Celano, M., Fenichel, R., Pollack, W. and Singher, H. *A "D-Like" Antigen in Rhesus Monkey, Human Rh Positive and Human Rh Negative Red Blood Cells*. J. Immunol. 87: 747-752
- 1962 Pollack, W., Hager, H.J., and Hollenbeck, L.L. Jr. *The Specificity of Anti-Human Gamma Globulin Reagents*. Transfusion 2: 17-30
- 1964 Freda, V.J., Gorman, J.G. and Pollack, W., *Successful Prevention of Experimental Rh Sensitization in Man with an Anti-Rh Gamma Globulin Antibody Preparation*. Transfusion 4: 26-32
- Levine, P and Pollack, W., *Hemolytic Disease of the Fetus and Newborn*. Med. Clin. of North America. 49: 1647-1660
- 1965 Pollack, W., *Some Physico-Chemical Aspects of Hemagglutination*. Ann. N.Y. Acad. Science 127: 892-900
- Pollack, W., Hager, H.J., Reckel, R., Toren, D.A., and Singher, H.O. *A Study of the Forces Involved in the Second Stage of Hemagglutination* Transfusion. 5: 158-153
- Robertson, J.G., Freda, V.J. and Pollack, W. *Prevention of Immunization to Rh with Anti- Rh Gamma Globulin*. Fed. Proc. 24: 696

- 1965 Cont'** Pollack, W. and Hager, H.J., *A Physico-Chemical Study of Hemagglutination*. Basel/New York; S. Karger. 10<sup>th</sup> Cong. Int. Soc. Blood Transfusion, Stockholm. 1964. pp 802-934
- Gorman, J.G., Freda, V.J., Pollack, W. *Prevention of Isoimmunization to Rh Factor Using High Titer Anti-Rh Gamma Globulin*. Basel/New York; S. Karger. Proc. 10th Cong. Int. Soc. Blood Transfusion, Stockholm. 1964. pp 949-955
- 1966** Freda, V.J., Gorman, J.G., Pollack, W., Robertson, J.G., Jennings, E.R. and Sullivan, J.F. *Prevention of Rh Isoimmunization*. J.A.M.A. 199: 390
- Gorman, J.G., Freda, V.J., Pollack, W. and Robertson, J.G. *Protection from Immunization in Rh-Incompatible Pregnancies. A Progress Report*. Bull. New York Acad. Med. 42: 458-473
- Pollack, W. *The Zeta-Potential and Hemagglutination with Rh Antibodies*. Manual for Workshop on the Techniques Used in Antibody Identification. Am. Assoc. Blood Banks.
- 1967** Pollack, W. and Tripodi, D. *The role of the Immune Response in the Establishment and Rejection of Neoplastic Tissue*. Int. Arch. Allergy and Immunol. 32: 429-436
- Freda, V.J., Gorman, J.G. and Pollack, W. *Suppression of Primary Rh Immune Response with Passive Rh Immune Globulin*. N.E.J.M. 277: 1022
- 1968** Pollack, W., Gorman, J.G., Freda, V.J., Jennings, E.R. and Sullivan, J.F. *Clinical Evaluation of Rh Immune Globulin in the Prophylaxis of Immunization to the Rh Factor*. Proc. 11th Cong. Int. Soc. Blood Transfusion, Sydney 1966 Bibl. Haemat. 29: 231-236
- Jennings, E.R., Dibbern, H.H., Hodell, F.H., Monroe, C., Peckham, N.H., Sullivan, J.F. and Pollack, W. *Long Beach ( California) Experience with Rh Immune Globulin*. Transfusion. 8: 146-148
- Pollack, W., Singher, H.O., Gorman, J.G. and Freda, V.J. *The Prevention of Isoimmunization to the Rh Factor by Passive Immunization with Rho(D) Immune Globulin (Human)*. Haematologia. 2: No.1: pp.1-8
- Pollack, W., Gorman, J.G. and Freda, V.J. *Prevention of Rh Hemolytic Disease*. Progress in Hematology. VII
- Pollack, W., Gorman, J.G., Freda, V.J., Ascari, W.Q. and Allen, A.E. *Results of Clinical Trials Of RhoGam in Women*. Transfusion. 8: 151-153

- 1969 Jennings, E.R., Dibbern, H.H., Hodell, F.H., Monroe, C.H., Peckham, N.H., Sullivan, J.F. and Pollack, W. *Prevention of Rh Hemolytic Disease of the Newborn*. California Medicine, 110:
- Ascari, W.Q., Levine, P. and Pollack, W. *Incidence of Maternal Rh Immunization by ABO Compatible and Incompatible Pregnancies*. Brit. Med. J. 1: 399-401
- 1970 Tripodi, D., Hollenbeck, L. and Pollack, W. *The effect of Endotoxin on the Implantation of a Mouse Sarcoma*. Int. Arch. of Allergy and Immunol. 37: 557-585
- Pollack, W. and Kochesky, R.J. *The Importance of Antibody Concentration Binding Constant and Heterogeneity in the Suppression of Immunity to the Rh Factor*. Int. Arch. Allergy and Immunol. 38: 410-415
- Pollack, W. and Reckel, R.P. *The Zeta-Potential and Hemagglutination with Rh Antibodies*. Int. Arch. of Allergy and Immunol. 38: 417-421
- 1971 Tripodi, D., Weis, J., Bonness, P.E. and Pollack, W. *Identification of Platelet Orosomucoid*. Transfusion 11: 139-144
- Pollack, W., Ascari, W.Q., Kochesky, R.J., O'Connor, R.R., Ho, T. and Tripodi, D. *Studies on Rhesus Prophylaxis with Rh Positive Blood*. No.1 Transfusion. 2: 333-339
- 1972 Pollack, W. *Rh Immune Globulin Administered to Du-Positive Mother: No Adverse Effect*. J.A.M.A. 221: No. 12
- Tripodi, D., Redeker, A., McKee, A.P., and Pollack, W. *Comparative Clinical Evaluation of H.A.A. Detection Among Liver Disease Patients*. Int. Arch. of Allergy and Immunol. 42: 681-685
- Tripodi, D., Gocke, D.J., McKee, A.P., Hawk, J., Campbell, J. and Pollack, W. *No.1. A comparative Clinical Evaluation of H.A.A Detection.No. 2. H.A.A Detection Among Blood Donors*. Transfusion. 12: 109-110
- 1973 Levine, P., Tripodi, D., Struck, J., Zmijewski, C.M. and Pollack, W. *Hemolytic Anemia Associated with Rh Null but not with Bombay Blood*. Vox. Sang. Suppl. 24: 80-83
- McKee, A.P., Pollack, W. and Tripodi, D. *Low Voltage Counterimmuno-electrophoreses for the Detection of Australia Antigen*. Vox. Sang. Suppl. 24: 80-83

- 1975** Freda, V.J., Gorman, J.G., Pollack, W. and Bowe, E. *Prevention of Rh Hemolytic Disease - Ten Years Clinical Experience with Rh Immune Globulin.* N.E.J.M. 293
- Rancourt, M.W., McKee, A.P. and Pollack, W. *Mitochondrial Profile of a Mammalian Lymphocyte.* Ultrastructure Res. 51: 418-424
- Redeker, A.G., Moseley, J.W., Gocke, D.J., McKee, A.P. and Pollack, W. *Hepatitis B Immune Globulin as a Prophylactic Measure for Spouses Exposed to Type B Hepatitis.* N.E.J.M. 293: 1055-1059
- 1976** Keith, L., Burger, G.S. and Pollack, W. *The Transfusion of Rh Negative Women.* Amer. J. Obs. Gyn. 125: 502
- Reiss, A.M. and Pollack, W. *Detection and Semiquantitation of Fetal Cells (Fetal Hemoglobin) in the Maternal Circulation.* Man. Clin. Immunol.
- Pollack, W. and Reckel, R.P. *A Reappraisal of the Zeta-Potential Model of Hemagglutination.* 5th Int. Convocation. Immunol., Buffalo, N.Y., 17-26
- 1977** Ratliff, E.A., Pollack, W., Tripodi, D. and Ho, T. *Solid Phase Radioimmune Assay for Quantitating Anti-HBs in Hepatitis B Immune Globulin.* Int. Arch. of Allergy and Immunol. 4: 231-237
- Pollack, W. and Reckel, R.P. *A Reappraisal of the Forces Involved in Hemagglutination.* Int. Arch. of Allergy and Immunol. 54: 231-237
- Freda, V.J., Gorman, J.G. and Pollack, W. *Prevention of Rh Hemolytic with Rh Immune Globulin.* Am. J. Obs. Gyn. 128: 456-460
- 1978** Gorman J.G., Rincon, F.J., Freda, V.J. and Pollack, W. *Closing the Utilization Gap on Rh Immune Globulin.* Med. Lab. Obs. March. p 199
- Freda, V.J., Pollack, W. and Gorman, J.G. *Rh Disease: How Near the End?* Hospital Practice. June. pp 61-69
- 1981** Pollack, W. *Rh Hemolytic Disease of the Newborn: Its Cause and Prevention.* Reproductive Immunology 70: 185-203
- Reckel, R.P., Harris, J., Botsko, E. and Pollack, W. *A new Capillary Tube Agglutination-Inhibition Test for the Detection of Circulating Immune Complexes Containing Endogenously Bound C1q.* Immunol. Letters. 3: 107
- 1982** Pollack, W. *Recent Understanding for the Mechanism by which Passively Administered Rh Antibody Suppresses the Immune Response to the Rh Antigen in Unimmunized Rh Negative Women.*

Clin. Obs. and Gyn. 25:255

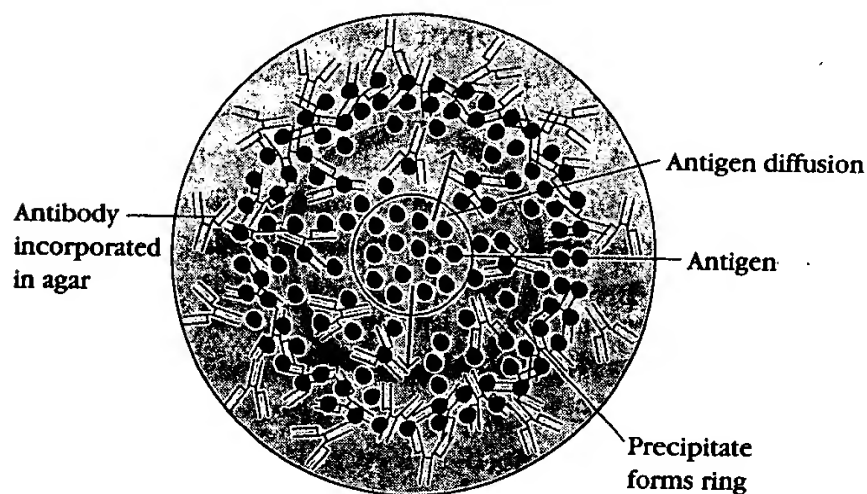
- 1984** Pollack, W. *Mechanism of Rh Immune Suppression by Rh Immune Globulin*. 37th Annual Meeting, A.A.B.B.. San Antonio. Texas.
- 1985** Pollack, W. and Iny, O. *A Physico-Chemical Study of PVPI Solutions Leading to Reformulation of Betadine Preparations ( 5% PVPI)*. J. Hosp. Infections 6: Supplement
- Gocke, D.J., Ponticas, S. and Pollack W. *In Vitro Studies of the Killing of Clinical Isolates by Povidone-Iodine Solutions*. J. Hosp. Infection 6: 59
- 1994** Pollack, W., Hogrefe, H. and Carstens, S.: *A Pre-Clinical Study of the use of "Blocking" Antibody Derived from Bee Keepers in the Prevention of Anaphylaxis in Seriously Allergic Patients*. J. Allergy and Appl. Immunol. (in preparation)
- 1995** Pollack, W. and Hogrefe, H.: *The Basophil Histamine Release Test as a Laboratory Surrogate for Patient's Allergic to Bee Venom*: In Preparation.
- Pollack, W. and Hogrefe, H.: *The Presence of Bee Venom "Blocking" Antibody in IgG4*: In Preparation.



## Precipitation Reactions in Gels

Immune precipitates can form not only in solution but also in an agar matrix. When antigen and antibody diffuse toward one another in agar or when antibody is incorporated into the agar and antigen diffuses into the antibody-containing matrix, a visible line of precipitation will form. As in a precipitation reaction in fluid, visible precipitation occurs in the region of equivalence, whereas no visible precipitate forms in regions of antibody or antigen excess. These **immunodiffusion reactions** can be used to determine relative concentrations of antibodies or antigens, to compare antigens, or to determine the relative purity of an antigen preparation. Two frequently used immunodiffusion techniques are **radial immunodiffusion** (Mancini method) and **double immunodiffusion** (Ouchterlony method).

### RADIAL IMMUNODIFFUSION



### DOUBLE IMMUNODIFFUSION

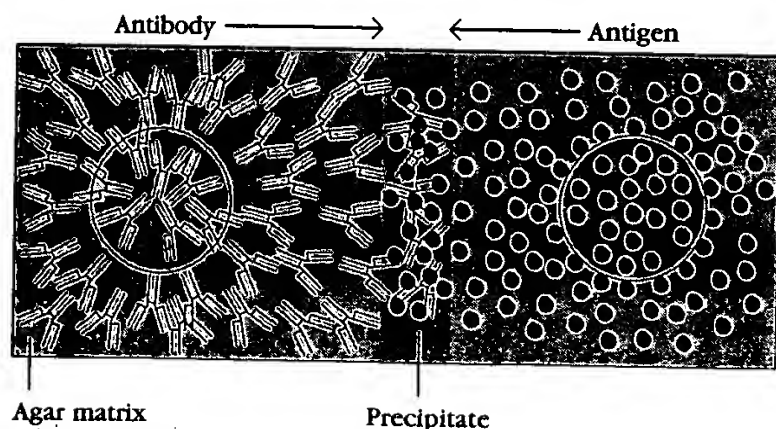


FIGURE 6-6

Diagrammatic representation of radial (Mancini) and double immunodiffusion (Ouchterlony) in a gel. In both cases, large insoluble complexes form in the agar in the zone of equivalence, which are visible as a line of precipitation (blue region). Only the antigen diffuses in radial immunodiffusion, whereas both the antibody and antigen diffuse in double immunodiffusion.

**diffusion** (Ouchterlony method); both are carried out in a semisolid medium like agar (Figure 6-6).

### RADIAL IMMUNODIFFUSION (MANCINI METHOD)

The relative concentrations of an antigen can be determined by a simple quantitative assay in which an antigen sample is placed in a well and allowed to diffuse into agar containing a suitable dilution of an antiserum. As the antigen diffuses into the agar, the region of equivalence is established and a ring of precipitation forms around the well (see Figure 6-6). The area of the precipitin ring is proportional to the concentration of antigen. By comparing the area of the precipitin ring with a standard curve (obtained by measuring the precipitin areas of known concentrations of the antigen), the concentration of the antigen sample can be determined.

The Mancini method is routinely used to quantitate serum levels of IgM, IgG, and IgA by incorporating class-specific anti-isotype antibody into the agar (Figure 6-7). The technique is also applied to determine the concentrations of complement components in serum. The Mancini method cannot detect antigens present in concentrations below 5–10  $\mu\text{g/ml}$ ; this moderate sensitivity is the major limitation of the radial immunodiffusion method.

### DOUBLE IMMUNODIFFUSION (OUCHTERLONY METHOD)

In the Ouchterlony method both antigen and antibody diffuse radially from wells toward each other, thereby establishing a concentration gradient. As equivalence is reached, a visible line of precipitation forms (see Figure 6-6). This simple technique is an effective *qualitative* tool for determining the relationship between antigens and the number of different Ag-Ab systems present.

The pattern of the precipitin lines that form when two different antigen preparations are placed in adjacent wells indicates whether or not they share epitopes (Figure 6-8):

- **Identity** occurs when two antigens share identical epitopes. The antiserum forms a single precipitin line with each antigen that grow toward each other and fuse to give a single curved line of identity.
- **Nonidentity** occurs when two antigens are unrelated (i.e., share no common epitopes). The antiserum forms independent precipitin lines that cross.
- **Partial identity** occurs when two antigens share some epitopes but one or the other has a unique epitope(s). The antiserum forms a line of identity with the common epitope(s) and a curved spur with the unique epitope(s).

# IMMUNOLOGY

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Interactions of cell adhesion molecules, with different ones involved at different times, are responsible for recruiting leukocytes to inflammatory sites and for their migration through the vascular endothelium. Slowed by vasodilation, leukocytes drift against vessel walls, where selectins are responsible for a loose adherence known as "rolling." This initial step in leukocyte migration is shown in a false-color scanning electron micrograph. (See Chapter 15 for more information.)

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